RULE-BASED MODELING FOR PROTEIN-PROTEIN INTERACTION NETWORKS - THE CYANOBACTERIAL CIRCADIAN CLOCK AS A CASE STUDY

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ABSTRACT

Rule-based modeling is a new approach to cope with the inherent combinatorial complexity in protein-protein interaction networks, such as cellular signaling pathways. In contrast to reactions that act on chemical species, rules can act on partially specified species. A single rule can thus account for multiple reactions and reflects the limited local context on which most protein-protein interaction events are conditioned on. The cyanobacterial circadian clock is purely protein-based and is centered around the cyclic phosphorylation of the hexameric KaiC protein. Its different phosphorylation states give rise to a combinatorial number of species that would be required for a traditional description. We give a detailed rule-based model, incorporating recent experimental findings of two-site phosphorylation and monomer exchange of the KaiC hexamer. Monte-Carlo sampling of kinetic parameters shows that monomer exchange alone may not be sufficient to synchronize the KaiC hexamers.

1. INTRODUCTION

Classically, protein-protein interactions are modeled using a set of ordinary differential equations derived from the principles of chemical kinetics. The theory of dynamical systems provides a rich methodology to analyze such models. For instance, the asymptotic behavior can be well determined without solving the equations explicitly. However, with the increasing amount of detailed knowledge about cellular signaling events, this approach faces fundamental limitations that relate to how chemical reactions are translated into such differential models. Proteins can bind and also modify each other through posttranslation modifications. This gives rise to a combinatorial number of protein complexes and modification states each of which needs to be considered as a new chemical species that associates with a new differential equation. For example, in the case study detailed below the central protein is a hexamer, each subunit of which carries two independent phosphorylation sites. Thus discarding symmetries this hexamer can exist in $4^6 = 4096$ different states that would give rise to as many distinct chemical species. A discussion on the combinatorial complexity encountered in other signaling systems is given in [1] and more recent analyses [2] reported astronomic numbers of 10^{19} different states in a model of the epidermal growth factor receptor (EGFR) pathway. The fact that this huge configuration space will in general be sparsely occupied, questions population dynamics as such. Besides the observation that proteins instead of chemical species appear to be more natural elementary units of description for such systems, the resulting huge equation systems are hard to interpret and modify.

This problem of traditional modeling is well recognized and people proposed heuristics to overcome it. One of them is to introduce aggregate variables that describe sets of modified forms of a particular molecule. For instance one might aggregate all phospho-forms of a receptor into one variable or just count the number of phosphorylated sites instead of enumerating its combinatorial configurations. Up to now this approach is unprincipled because it does not rely on a microscopic description that could support such simplifications.

Recent attempts to solve these problems are made by describing biochemical networks in an agent-based framework. A molecule is represented as an agent whose possible interactions are defined by rules that specify how its binding configuration and its internal states (e.g. phosphorylation) change. The framework exploits the fact that many chemical events are *local* in the sense that they are conditioned only on a small subset of states in the state space. For instance, the phosphorylation of a protein through a kinase may not depend on the fact that this protein is bound to another protein.

The observation that signaling pathways are massively distributed, concurrent systems has led Regev et al. [3] to propose Milner's π-calculus [4] for their description. Since then, numerous variants of this calculus focusing on different modeling situations have been developed. Pathways for which such types of models have been designed include MAPK cascades, the EGFR pathway, the yeast mating pheromone pathway and others (see [1, 2] and the references therein). Such models allow to enumerate only that context of a chemical event that was reported necessary by experiments.

2. KAPPA - A RULE-BASED LANGUAGE

Among the various attempts to tailor process algebra formalisms to biology, the rule-based language Kappa [2] is a promising one. Kappa is a context-free formal language to define agents (normally meant to be proteins) and rules of interactions. Given are a countable set of agent names $\mathcal{A}$, a countable set of site names $\mathcal{S}$, corresponding to domain and residues of proteins, a finite set of internal states $\mathcal{M}$ and a set of bond labels $\mathcal{B}$. An interface is a set of sites with their internal and binding states. An agent a is given by a name in $\mathcal{A}$ and an interface. Following [2] we define

agents below using the Backus-Naur notation, a metasyntax to describe context-free grammars.

Agent	$a ::= N(\sigma)$	(1)
Agent name	$N ::= A \in \mathcal{A}$	(2)
Interface	$\sigma ::= \emptyset \vert \sigma, s$	(3)
Site	$s ::= n_\iota^\lambda$	(4)
Site name	$n ::= x \in \mathcal{S}$	(5)
Internal state	$\iota ::= \epsilon \,\vert\, m \in \mathcal{M}$	(6)
Binding state	$\lambda ::= \epsilon \,\vert\, - \,\vert\, ? \,\vert\, i \in \mathcal{B}$	(7)

According to (7) the binding status λ can be specified in four different ways, i.e., either free ($\lambda = \epsilon$), bound to something unspecified ($\lambda = -$), bound to link $\lambda = i$ with $i \in \mathcal{B}$ or binding state not specified at all ($\lambda =?$). In the same way an internal state can either be unspecified ($\iota = \epsilon$) or specified ($\iota = m$ with $m \in \mathcal{M}$). The state specifier ϵ can be thought of a wildcard with the convention $A(x_\epsilon^\epsilon) \equiv A(x)$. Furthermore, the convention $A(x, y_\epsilon^?) \equiv A(x)$ applies, i.e., if nothing is specified for a site, the site is omitted and yields a partially specified interface of agent A.

An object that consists of a collection of agents (bound or unbound) is referred to as an *expression*. Expressions can represent protein complexes. We define an expression as

$$E ::= \emptyset | a, E \tag{8}$$

A rule is a pair of expressions. It transforms its left-hand-side (lhs) expression into its right-hand-side (rhs) expression. Note, that an expression may contain agents with a partially specified interface σ, i.e., sites are omitted or their internal or binding status remain unspecified. This is precisely the feature that makes rule-based models circumvent the combinatorial explosion that is encountered in classical chemical kinetics.

3. CIRCADIAN CLOCK OF CYANOBACTERIA

The purpose of the circadian clock in cyanobacteria is to regulate gene expression mainly in order to alternate between the exclusive processes of nitrogen fixation and photosynthesis according to light availability. During the last ten years, the cyanobacterial circadian clock has been intensively studied. In 2005 a landmark experiment was conducted [5], where Kais oscillations were reproduced *in vitro* by mixing the proper amount of three Kai proteins with ATP. The experimental proof that only three proteins are sufficient to sustain the oscillations triggered many attempts to model this system. The incorporation of recent experimental findings about the structure of these proteins into such models, gave rise to a combinatorial increase in model complexity. For instance a recent model of this clock involves as much as 24,576 different states [6].

3.1. The Kai protein family

Three proteins called KaiA, KaiB and KaiC are the core constituents of the cyanobacterial circadian clock. These proteins when isolated and mixed in the correct concentrations and with ATP start to oscillate: KaiC is periodically phosphorylated, reproducing the circadian cycle in vitro [5]. These proteins have relations with other proteins and their expression is controlled, but in our research we are only interested in the in vitro system; we will not discuss the transcription/translation processes related to these proteins.

The first two proteins, KaiA and KaiB, act on the phosphatase activity of KaiC. Their interactions with KaiC are controlled by the phosphorylation state of KaiC. In its active state, KaiA is always present as a dimer and we consider it as such in our model. One KaiA dimer binds to one KaiC hexamer [7] independently of its phosphorylation state [8]. KaiB forms dimers. Detailed studies showed that KaiB most likely associated with KaiC in form of two dimers (which we consider as a tetramer) in a ring shape that attaches on top of the KaiC hexamer [9]. KaiB binds to the KaiC hexamer only when it is in a high-phosphorylated state [10].

KaiC gene is a duplicate version of a recA/dnaB-like gene [11]. RecA is a DNA recombinase and DnaB is a DNA helicase; their similarity with KaiC implies that KaiC may also act upon DNA. The half-sites of KaiC (CI for N-terminal and CII for the C-terminal) contain shared regions that include Walker A and B motifs involved in ATP binding and hydrolysis [11]. The CII half of the KaiC hexamer has at least two phosphorylation sites: a serine (S) at position 431 and a threonine (T) at 432 [10]. A substitution of any of those sites with alanine (correspond to the non-phosphorylated state) results in a complete loss of rhythmic activity [12].

KaiC was observed to have a weak autophosphorylation and a stronger autodephosphorylation activity. KaiA was observed to enhance the autophosphorylation activity of KaiC and inhibits KaiC's autodephosphorylation tendency [10]. Without KaiA, fully phosphorylated KaiC hexamers dephosphorlate competely within 24h [8]. The effect of KaiB when attached to KaiC is to inhibit KaiA phosphorylation effect and trap KaiA until the full dephosphorylation of KaiC. KaiB alone does not have a effect on KaiC (de-)phosphorylation activity [9, 10].

Recent experiments [10] showed that there is a strict sequential program for the phosphorylation and dephosphorylation of the S and T site on KaiC. Stating from the fully unphosphorylated state (Su/Tu), first T is phosphorylated (Su/Tp) with the help of KaiA and then S (Sp/Tp). At this stage only, KaiB can attach KaiC and block KaiA effect resulting in a dephosphorylation of T (Sp/Tu) and finally of S (Su/Tu).

The last important fact about KaiC is the possibility for monomers to be exchanged between hexamers. This mechanism of shuffling occurs in the early dephosphorylation stage [8, 12]. It has been postulated as a necessary and sufficient mechanism to synchronize the population of KaiC hexamers and maintain coherent oscillations of large amplitude [6, 12] for the experimentally observed period of time.

4. KAPPA MODEL OF THE CORE CLOCK

Apart from illustrating the use of Kappa the main purpose of the presented models is to test a parsimonious model, the accounted interactions of which are experimentally well established. The question is whether such a mini-

mal model is sufficient the explain the experimental data, in particular the long coherence time and amplitude of the oscillations. The model comprises the following facts and interactions: (1) each KaiC monomer has two phosphorylation sites, therefore four different states (Su/Tu, Su/Tp, Sp/Tp, Sp/Tu) [13, 10]; (2) KaiC monomers form hexamers, but monomer units can detach from hexamers and enter other complexes lacking monomers. Monomers can detach and attach only during the dephosphorylation phase when KaiB is bound to the KaiC complex [10]; (3) KaiA is modeled as a dimer. One KaiA dimer can bind to one KaiC hexamer [7] and detach if no KaiB is bound [10]. If only KaiA is bound, autophosphorylation activity of KaiC is enhanced [14]; (4) KaiB is modeled as a tetramers. One KaiB tetramer can bind to a high-phosphorylated KaiC hexamer [9] and detach when KaiC is in a low-phosphorylated state. KaiB reverts the effect of KaiA on KaiC autophosphorylation rate [9].

In order to define context-free reactions on the monomer units of the KaiC hexamer and allow for monomer exchange, we introduce a virtual scaffold protein

$$\mathtt{VScf}(\mathtt{c},\mathtt{c},\mathtt{c},\mathtt{c},\mathtt{c},\mathtt{a},\mathtt{b}),$$

from which monomers can dissociate and re-associate. The interface σ of VScf consists of six non-distinguishable binding sites (i.e. we allow the interface to be a multiset) for the monomeric KaiC proteins and two binding sites for KaiA and KaiB. We denote the corresponding agents in the Kappa model as, $\mathtt{KaiC}(\mathtt{v},\mathtt{s},\mathtt{t})$, $\mathtt{KaiA}(\mathtt{v})$ and $\mathtt{KaiB}(\mathtt{v})$, respectively. The rule-based model starts with the association of KaiA to the KaiC hexamer VScf and the trapping of the former in the presence of KaiB.

$$\begin{aligned}
\mathtt{VScf}(\mathtt{a}),\mathtt{KaiA}(\mathtt{v}) &\xrightarrow{\gamma_1} \mathtt{VScf}(\mathtt{a}^1),\mathtt{KaiA}(\mathtt{v}^1)\\
\mathtt{VScf}(\mathtt{a}^1,\mathtt{b}),\mathtt{KaiA}(\mathtt{v}^1) &\xrightarrow{\gamma_2} \mathtt{VScf}(\mathtt{a},\mathtt{b}),\mathtt{KaiA}(\mathtt{v})\\
\mathtt{VScf}(\mathtt{a}^1,\mathtt{b}^-),\mathtt{KaiA}(\mathtt{v}^1) &\xrightarrow{\gamma_3} \mathtt{VScf}(\mathtt{a},\mathtt{b}^-),\mathtt{KaiA}(\mathtt{v}),
\end{aligned}$$

where for the dissociation a *kinetic refinement* is made in order to account for the observed trapping of KaiA through KaiB, i.e., $\gamma_2 > \gamma_3$. Next, we account for the observed strict sequential order [10] in the phosphorylation of the two sites per KaiC monomer

$$\begin{aligned}
&\mathtt{VScf}(\mathtt{s}^1,\mathtt{a}^-,\mathtt{b}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u},\mathtt{t}_\mathtt{u})\\
&\qquad\xrightarrow{\gamma_4} \mathtt{VScf}(\mathtt{s}^1,\mathtt{a}^-,\mathtt{b}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u},\mathtt{t}_\mathtt{p})\\
&\mathtt{VScf}(\mathtt{s}^1,\mathtt{a}^-,\mathtt{b}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u},\mathtt{t}_\mathtt{p})\\
&\qquad\xrightarrow{\gamma_5} \mathtt{VScf}(\mathtt{s}^1,\mathtt{a}^-,\mathtt{b}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{p}),
\end{aligned}$$

with $\mathcal{M} = \{u, p\}$ throughout. Note that this rule is local in the sense that it just involves a single monomer of the hexameric KaiC protein. For the association of KaiB to the KaiC hexamer we have the stringent condition that all subunits need to be doubly phosphorylated. Due to the above sequential order it is sufficient to only mentioned the site s of KaiC

$$\begin{aligned}
&\mathtt{VScf}(\mathtt{c}^1,\ldots,\mathtt{c}^6,\mathtt{b}),\\
&\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p}),\ldots,\mathtt{KaiC}(\mathtt{v}^6,\mathtt{s}_\mathtt{p}),\mathtt{KaiB}(\mathtt{v}) \xrightarrow{\gamma_6}\\
&\mathtt{VScf}(\mathtt{c}^1,\ldots,\mathtt{c}^6,\mathtt{b}^7),\\
&\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p}),\ldots,\mathtt{KaiC}(\mathtt{v}^6,\mathtt{s}_\mathtt{p}),\mathtt{KaiB}(\mathtt{v}^7).
\end{aligned}$$

The association of KaiB is thus unconditional with respect the binding status of KaiA. Dissociation of KaiB occurs only after dephosporylation of all subunits

$$\begin{aligned}
&\mathtt{VScf}(\mathtt{c}^1,\ldots,\mathtt{c}^6,\mathtt{b}^7),\\
&\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u}),\ldots,\mathtt{KaiC}(\mathtt{v}^6,\mathtt{s}_\mathtt{u}),\mathtt{KaiB}(\mathtt{v}^7) \xrightarrow{\gamma_7}\\
&\mathtt{VScf}(\mathtt{c}^1,\ldots,\mathtt{c}^6,\mathtt{b}),\\
&\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u}),\ldots,\mathtt{KaiC}(\mathtt{v}^6,\mathtt{s}_\mathtt{u}),\mathtt{KaiB}(\mathtt{v}),
\end{aligned}$$

where we exploited the fact that dephosphorylation also follows a strict sequential order [10]. While KaiB is bound we allow for monomer exchange

$$\mathtt{VScf}(\mathtt{c}^1,\mathtt{b}^-),\mathtt{KaiC}(\mathtt{v}^1) \underset{\gamma_9}{\overset{\gamma_8}{\rightleftharpoons}} \mathtt{VScf}(\mathtt{c},\mathtt{b}^-),\mathtt{KaiC}(\mathtt{v}).$$

Finally we need to define the autodephosphorylation of the KaiC subunits when KaiA's action is inhibited by KaiB

$$\begin{aligned}
&\mathtt{VScf}(\mathtt{c}^1,\mathtt{b}^-,\mathtt{a}^-),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{p})\\
&\qquad\xrightarrow{\gamma_{10}} \mathtt{VScf}(\mathtt{c}^1,\mathtt{b}^-,\mathtt{a}^-),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{u})\\
&\mathtt{VScf}(\mathtt{c}^1,\mathtt{b}^-,\mathtt{a}^-),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{u})\\
&\qquad\xrightarrow{\gamma_{10}} \mathtt{VScf}(\mathtt{c}^1,\mathtt{b}^-,\mathtt{a}^-),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u},\mathtt{t}_\mathtt{u})
\end{aligned}$$

and when no KaiA is bound to the hexamer

$$\begin{aligned}
&\mathtt{VScf}(\mathtt{c}^1,\mathtt{a}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{p})\\
&\qquad\xrightarrow{\gamma_{11}} \mathtt{VScf}(\mathtt{c}^1,\mathtt{a}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{u})\\
&\mathtt{VScf}(\mathtt{c}^1,\mathtt{a}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{u})\\
&\qquad\xrightarrow{\gamma_{11}} \mathtt{VScf}(\mathtt{c}^1,\mathtt{a}),\mathtt{KaiC}(\mathtt{v}^1,\mathtt{s}_\mathtt{u},\mathtt{t}_\mathtt{u}).
\end{aligned}$$

A free floating monomer also autodephosphorylates with

$$\begin{aligned}
\mathtt{KaiC}(\mathtt{v},\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{p}) &\xrightarrow{\gamma_{12}} \mathtt{KaiC}(\mathtt{v},\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{u})\\
\mathtt{KaiC}(\mathtt{v},\mathtt{s}_\mathtt{p},\mathtt{t}_\mathtt{u}) &\xrightarrow{\gamma_{12}} \mathtt{KaiC}(\mathtt{v},\mathtt{s}_\mathtt{u},\mathtt{t}_\mathtt{u}).
\end{aligned}$$

Discarding symmetries, this model generates $39,448$ reachable complexes or species.

5. RESULTS

In order to test whether this parsimonious model can exhibit persistent large-amplitude oscillations, we randomly sampled parameter vectors $(\gamma_1,\ldots,\gamma_{12},\varrho)$ within an interval $\gamma_j \in [10^{-2},10^2]$ (per hour or per hour per molecule) for all j and with ϱ the ratio between KaiA dimers and KaiC hexamers that was reported to be an important control parameter [8]. In order to reduce the dimensionality of the sampling we assumed $\gamma_3 = 0.01$, $\gamma_4 = \gamma_5$ and $\gamma_{10} = \gamma_{11} = \gamma_{12}$. To assess period and amplitude of oscillations in the number of phosphorylated KaiC subunits we performed stochastic simulations. We used $2400 \times$ KaiC monomers, $\lceil 400\varrho \rceil \times$ KaiA dimers and $1200 \times$ KaiB tetramers. Figure 1 shows two-dimensional projections of the parameter volume and indicate for each parameter sample the corresponding quality-score of oscillation in terms of amplitude and period using gray-level coding. These particular projections were chosen because they exhibit a clear trend and correlation. The score is determined by counting how many periods in sample path

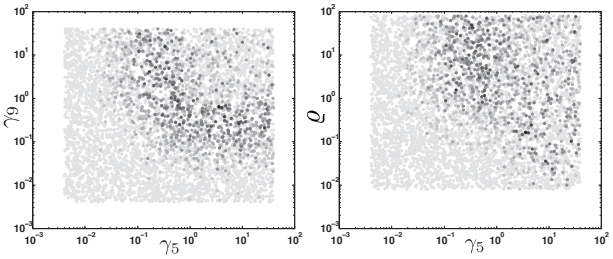

Figure 1. Two-dimensional projections of the 13-dimensional parameter volume; darker gray-level indicating higher quality-score of oscillations.

from a stochastic simulation for a given parameter vector comply with the criteria. The criteria were loose in the sense that we required a period within $[10, 30]$ hours and a peak amplitude of at least 50 phosphorylated `KaiC` monomers bound within a hexamer. From the projections it is evident that a trend in parameter space exists and a region of larger oscillation amplitude can be identified. However, the relative oscillation amplitude with respect to the total `KaiC` copy number for the best parameter set is significantly below the experimentally observed one [5].

6. CONCLUSION

We presented a parsimonious mechanistic model of the cyanobacterial circadian oscillations. By deploying the context-free formalism of rule-based modeling we are able to concisely and precisely define and execute this very combinatorial model. The observed monomer exchange between hexamers during the dephosphorylation phase has been conjectured to be a sufficient mechanism for synchronization of individual KaiC hexamers. We show first evidence, that in a detailed mechanistic model it may *not* be sufficient. We randomly sampled parameter sets within a wide range and assessed their corresponding oscillation through stochastic simulation for a realistic population size. The obtained maximal oscillation amplitude was far below the experimentally observed one. This may hint to the necessity for a even more ordered phosphorylation cycle, perhaps to due the reported subunit interactions in the KaiC hexamers [14].

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